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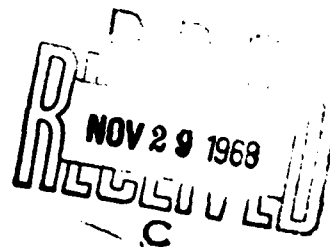
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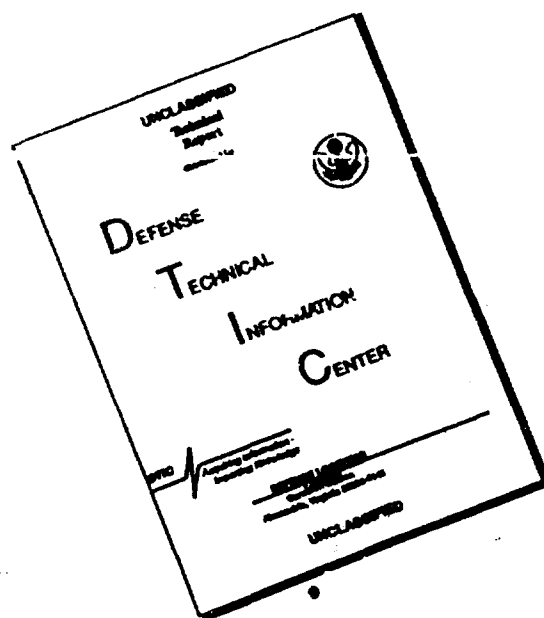
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RESPIRATION OF FRANCISELLA TULARENSIS GEOGRAPHICAL
VARIANTS IN THE PRESENCE OF GLYCERIN

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i Immunobiologii (Journal of Micro-
biology, Epidemiology and Immuno-
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At the present time two geographical variants of the tularemia microbe are distinguished: palearctic and nearctic. The palearctic variant is relatively less pathogenic for humans and domestic rabbits and does not ferment glycerin. The nearctic variant is highly pathogenic for domestic rabbits, causes a more serious course of tularemia in humans, and ferments glycerin. The different behavior toward glycerin of the variants indicated has been established by Olsuf'yev and associates (Olsuf'yev and coauthors, 1959; Olsuf'yev and Yemel'yanova, 1962; Yemel'yanova, 1964; Yemel'yanova and coauthors, 1965) by the Downs method, i. e. upon cultivating the microbe on a solid nutrient medium containing glycerin. The assimilation of glycerin was judged by the color change of an indicator as a result of the formation of acid fermentation products. The work of Skrodzki and coauthors (1963), which compared the biochemical activity of different strains of the tularemia microbe by the respirometric method in the Warburg apparatus, merits attention. The investigations showed that the Newton and Schu strains, which belong to the nearctic variant of the microbe, differed from the palearctic strains (No. 503, Ig, SD and 118) by intensive oxidation of glycerin.

It was of interest to check by this more accurate method the behavior toward glycerin of various tularemia microbe strains belonging to both variants which were isolated from different sources in different countries of Eurasia and on the American continent and which vary in degree of virulence for laboratory animals.

We studied 35 strains. Twenty-eight strains isolated in Europe and Asia and two strains isolated in the USA — KF and B-500 — belonged to the palearctic variant. The remaining strains isolated in the USA belonged to

the nearctic variant. Some strains lost virulence even for mice as a result of attenuation under the conditions of laboratory cultivation, but such cultures maintained their behavior toward glycerin upon determination by the Downs method (see table).

A two-day-old culture incubated at 37° on a medium of fresh fish hydrolysate with cystine and glucose, washed off twice and brought to a concentration of 10^{10} cells in one ml with physiological solution in accordance with the State Control Institute standard was used for the investigation.

The intensity of respiration was determined by the direct method in the Warburg apparatus (Umbreyt and coauthors, 1951) at 37° (air as the gas phase). Into the main section of the Warburg vessel were introduced 1.5 ml 0.1 M phosphate buffer, pH 7.4, and one ml of the microbe suspension; into the side section — 0.5 ml 0.15 M DL-glycerin in 0.1 M phosphate buffer, pH 7.0, or the buffer referred to without glycerin; in the center small beaker — 0.2 ml 10 percent KOH. After 15 minutes equilibration the substrate was added to the suspension, and the endogenous respiration, i. e. the respiration due to intracellular substrates, was measured in a parallel manner. The manometer readings were taken every 10 minutes for an hour; the oscillation frequency was 120 per minute.

Bacterial nitrogen after mineralization was determined by the Conway method (1942).

The intensity of respiration of the microbes is expressed by the coefficient $Q_{O_2}(N)$ (number of microliters of oxygen used up in an hour per one mg bacterial nitrogen). The respiration due to the glycerin added (substrate respiration) was calculated as the difference between the total (in the presence of glycerin) respiration and the endogenous respiration.

The virulence of the strains toward white mice and domestic rabbits was checked at the time of carrying out the investigation.

The results of the investigation are given in the table. The data of Olsuf'yev and associates, obtained by them by the Downs method, are included in the table for comparison.

The strains were divided into three groups according to the amount of substrate respiration: one with $Q_{O_2}(N)$ values from 0 to 10, which is within the limits of error of the method; one with values from 10 to 100, which testifies to weak or moderate respiration, and one with values over 100, which characterizes extremely intensive respiration.

The Schu, No. 8859, No. Q-284, Schu-avirulent and No. 38 strains of the nearctic variant were distinguished by the most intensive substrate respiration. The $Q_{O_2}(N)$ reading for them varied from 546.48 to 484.05 in the first experiment and from 207.66 to 555.75 in the second. We did not discover any substantial differences between the virulent and avirulent strains

Absorption of Oxygen by Cell Suspensions of Various Tularemia Microbe Strains

1)		2)		3)		4)		5)		6)		7)		8)		9)	
Рассеянность		Штамм		Место выделения		Источник выделения		100 % спорозави- днее при микроскоп- ном исследовании (в микро- пипетках) для		Бактери- цидная актив- ность		Ферментатив- ная актив- ность		Q ₁ (M) (в мл/мл N бактерий за 1 час)			

Legend on following page

- LEGEND:**
- 1) Variant
 - 2) Strain
 - 3) Locality of isolation
 - 4) Isolation source
 - 5) 100 percent lethal dose upon subcutaneous inoculation (in microbe cells) for:
 - 6) white mice
 - 7) rabbits
 - 8) Glycerin fermentation, determined by Downs method**
 - 9) $QO_2(N)$ (in $\mu l/mg$ of bacteria in 1 hour)
 - 10) Experiment I
 - 11) Experiment II
 - 12) Endogenous
 - 13) Substrate
 - 14) T'ung-Liao
 - 15) (reduced)*
 - 16) avirulent

<u>Column 1)</u>	<u>Column 3)</u>	<u>Column 4)</u>
Paleartic	USA	Field mouse
		Beaver
	France	Hare
	Italy	"
	Yugoslavia	Wood mouse
		Hare
	Bulgaria	Muskrat
	China	Marmot
	USSR	Ticks
		Field mouse
		Water rat
		Reddish-gray mouse
		Wood mouse
		Red mouse
		Brook
		Reservoir
Nearctic	Japan	Man
		"
		Ticks
		Man
		?
		?
		Man
		Colt
		Ticks
		Man
	USA	

Notes on following page

Notes: Three dots — investigation not carried out.

* Attenuated under laboratory conditions.

** According to data of G. Olsuf'yev and associates.

*** Isolated in Khabarovskiy Kray.

**** Isolated on Sakhalin.

within this group in the ability to assimilate glycerin. For Schu-avirulent and No. 38 strains attenuated under laboratory conditions the $Q_{O_2}(N)$ values were of the same order of magnitude as for the virulent ones, being distinguished from them only by a weaker endogenous respiration; the endogenous respiration of strain No. 38 could not be ascertained by means of the method used.

Of the 30 palearctic strains the six isolated in Japan — Kosho, Sudo, Jama, Tsushiya, Ebina and attenuated TH — revealed moderate substrate respiration. The indices of respiration due to the glycerin varied for them in the 30-100 range. On determination by the Downs method only two of them — Kosho and Sudo — assimilated glycerin.

In spite of the geographical proximity of the Japanese strains and the seven Sakhalin strains, only one of the latter — No 91/3 — proved to be able to oxidize glycerin.

The remaining 17 palearctic strains, including the attenuated 15-reduced and No. 21/400 ones, did not use oxygen for the oxidation of glycerin, which agrees with the results of the experiments of fermentation.

Endogenous respiration was highest in virulent strains of the palearctic variant isolated in various countries of the American and Eurasian continents, excluding Japan and Sakhalin. Among this group of palearctic strains the avirulent No. 21/400 strain, like the Schu-avirulent and No. 38 strains from the nearctic group, was distinguished by low endogenous respiration. Thus, among the palearctic group the Japanese strains and perhaps one of the Sakhalin strains, which displayed a rather considerable ability to oxidize glycerin in contrast to the other strains of this group, are outstanding.

On the basis of the data obtained we consider it possible to distinguish in the palearctic variant of the tularemia microbe a Japanese variant Francisella tularensis palaeartica var. japonica, Rodionova, distinguished from the typical palearctic strains by the ability to oxidize glycerin

[Note: After the present article was sent to press an article by Sato and coauthors, "Glycerol Fermentation of Francisella tularensis" (Ann. Rep. Chara Hosp., Vol 10/1, N 16, 1966, pp 10-13) was published. The article is devoted to a study of the fermentation of glycerin by the Downs method by 89 strains of the tularemia microbe, of which 76 were isolated in Japan, 10 in America, 2 in the USSR and 1 in Austria. The Japanese scientists' results completely agree with our data. Just like the typical palearctic variant,

the Japanese variant does not contain the enzyme citrullinureidase, unlike the American (nearctic) variant. This question will be treated in greater detail in a special report.

CONCLUSIONS

1. The strains studied which belong to the nearctic variant of the tularemia microbe oxidised glycerin very actively.
2. The strains of the palearctic variant, excluding the Japanese ones, are not able to oxidise glycerin. The latter revealed a marked capacity for respiration due to this substrate.
3. Upon attenuation of the strains of both variants the ability to oxidise glycerin was maintained at the level characteristic for the variant in question.
4. The Japanese strains represent a variant of the palearctic variant of the tularemia microbe which oxidizes glycerin — Francisella tularensis palearctica varietas japonica, Rodionova, 1966.

Submitted to the editors
9 February 1966

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